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USE OF A SIMPLE EXPERIMENTAL DEVICE TO STUDY WATER POLLUTION IN SITU: COMPARATIVE EFFECTS OF THREE ANTI-PETROLEUM EMULSIVE AGENTS

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USE OF A SIMPLE EXPERIMENTAL DEVICE TO STUDY WATER POLLUTION IN SITU: COMPARATIVE EFFECTS OF THREE ANTI-PETROLEUM EMULSIVE AGENTS

[Utilisation d'un dispositif expérimental simple pour l'étude de la pollution des eaux in situ: effets comparés de trois agents émulsionnants anti-pétrole]

By

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Abstract

By establishing in situ experimental ecosystems, it was possible to conduct pollution research under actual conditions. The experiments were carried out in an oyster culture basin of a lagoon in the Sables d'Olonne region by using devices to section off the water into a number of similar natural environmental compartments to be used for pollutant treatments and controls. Each device isolated 560 ℓ of water and consisted of a polyethylene sheath equipped with 60-cm diameter polyvinyl chloride rings. Floats were attached to the upper rings, while the lower ones were weighted and became buried in the sand. With the exception of one control ecosystem, each of these experimental ecosystems received a pollutant in the form of an anti-petroleum

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product at a rate of 40 mg/ℓ. The criterion used to illustrate the effects of pollution were photosynthetic activity, concentration of chlorophyll *a* and dry weight of plankton.

The product Corexit produced only weak and temporary changes. Sefoil acted as a fertilizer, provoking a rapid increase in photosynthesis and chlorophyll a concentration; this was followed by considerable growth in the zooplankton, particularly the copepods, which resulted in the disappearance of the phytoplankton by the end of the experiment. The surface-active fraction of the third product used, Gamosol, caused an almost complete inhibition of photosynthesis during the entire 15 days of the experiment.

* * *

1. Introduction

It is difficult to apply the results of toxicological tests, which are usually performed on isolated species in the laboratory, to the natural environment. Additional tests must also be performed on natural ecosystems in order to work with actual pollution conditions. In this way it is possible to compare areas treated with a pollutant with control areas. Tests of this type were performed in an oyster culture basin in the Sables d'Olonne region by sectioning similar portions of the natural environment with the use of transparent polyethylene film. It was necessary to meet several conditions in order actually to perform these bioassays in situ the experimental devices must be of simple design, easy to handle, inexpensive, and easy to construct, and should produce very little change in the natural environment.

II. Equipment and Method

1) Experimental Device

For the past 10 years, transparent polyethylene film has been used in the fields of oceanography and limnology to isolate relatively large quantities of water (McAllister et al., 1961; Stepanek and Zelinka, 1961; Strickland and Terhune, 1961; Goldman, 1962; Gachter, 1968, Kemmerer, 1968, 1969). With the exception of Goldman (1963), however, few investigators have used these devices for pollution studies.

Obviously, the preparation of an experimental device depends on the type of biotope one intends to study; the device described here is designed to be used in continental water of slight depth (a few meters) over a bottom layer of loose sediments.

Our tests were performed in June 1970, in an oyster culture basin, the "Chasses Basin," in a lagoon. A canal connects this basin to a series of coastal ponds collectively termed the "Olonne Marsh." The "Chasses Basin" is also in direct communication with the ocean through the Chaume locks, located in the outer harbor of Sables d'Olonne. The water in this basin is periodically renewed. Since this renewal occurred every 15 days during our experiments, we limited them to this period of time.

The experimental devices we prepared consisted of transparent polyethylene sheaths equipped with polyvinyl chloride rings 60 cm in diameter (Fig. 1). Expanded polystyrene floats were affixed to the surface ring, while the opposite ring, which was weighted, became buried in the sediments to about 5 cm. An intermediate ring kept the sheath in a cylindrical shape. The sheath , 25/100 mm thick, covered the internal surfaces of the rings. It was folded back after passing through the surface and bottom rings. All the

sheath-ring connections were obtained with highly adhesive polyethylene tape. This tape was also used to reinforce the sheath in areas where it would be subjected to friction or stress. The floats were positioned at the lower third of surface ring by means of small nylon cords attaching them along a row of perforations. Since each device sectioned a surface area of 0.20 m^2 at a depth of 2 m, each one enclosed approximately 560 ℓ of water.

Eight experimental devices were submerged. One of these was used as a control, and the three others were polluted with an anti-petroleum product at a concentration of 40 mg/ℓ each. The three products used were Gamosol, Sefoil and Corexit. Three main parameters were used to compare their effects: rate of photosynthesis, chlorophyll a concentration and dry weight of plankton. The four remaining devices respectively received 1, 5, 10 and 20 mg/ℓ of a nonionic detergent (nonylphenol with nine ethylene oxides) included in the composition of one of the anti-petroleum products (Gamosol). In the latter cases, the pollutant had begun to influence the rate of photosynthesis of the enclosed water 4 days after the beginning of the experiment.

2) Composition of Pollutants

Gamosol[1]. This emulsive agent was spilled in massive quantities in the wreck of the oil tanker the Torrey Canyon on March 18, 1967. This compound, with an average density of 0.92, is a mixture of nonionic surface-active products and aromatic solvents (Anonymous, 1967; Gillerot and Cadron, 1967). The surface-active compounds which it contains are derived from the same initial hydrophobe material, nonylphenols (trade name for a mixture of

[1] Gamlen and Naintré Company.

monoalkylphenol isomers), but differing as to the quantity of hydrophilic components (ethylene oxides). By increasing the proportion of these components, different hydrophile-hydrophobe ratios may be obtained. These surface-active compounds have a number of ethylene molecules, ranging from four to nine. Gamosol consists of 14% nonylphenols, with solvents making up the remaining 86%. These solvents are aromatic hydrocarbons (substituted benzenes) comprising a residue from kerosene distillation. Our research was limited to study of the effects of the polyethylenated nonylphenol fraction of Gamosol, since the solvent fraction, which is extremely toxic (Lacaze, 1967; Lacaze, forthcoming), is quickly eliminated in a dilute solution. This elimination is also a function of the aeration of the environment (Lacaze, 1969). We have made a more detailed study of the effects of one of the surface-active agents in Gamosol, nonylphenol with nine ethylene oxides (Syntopon C)[1], which is also used for domestic purposes. It should be pointed out that, of the many types of nonionic compounds, alkylphenolpolyoxyethylenes with varying numbers of ethylene oxide groups -- especially octylphenols, nonylphenols and dodecylphenols -- are consumed in the largest quantities. Thus it is not surprising that nonionic surfactants now play a substantial role in water pollution. (Thus Vadot noted concentrations on the order of 1 mm/ℓ in the liquid waste from the city of Lyon in 1967).

Sefoil.[2] Anti-petroleum products of the type mentioned above, which are especially dangerous, cannot be used in the sea to treat large areas. We therefore became interested in products with a completely different composition, such as Sefoil (Fusey et al., 1969). This product, with a density of

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[1] We would like to thank Witco Chemical Company, which was kind enough to supply us with this product in a form consisting of 100% active ingredients.
[2] Patented by G. Salomone.

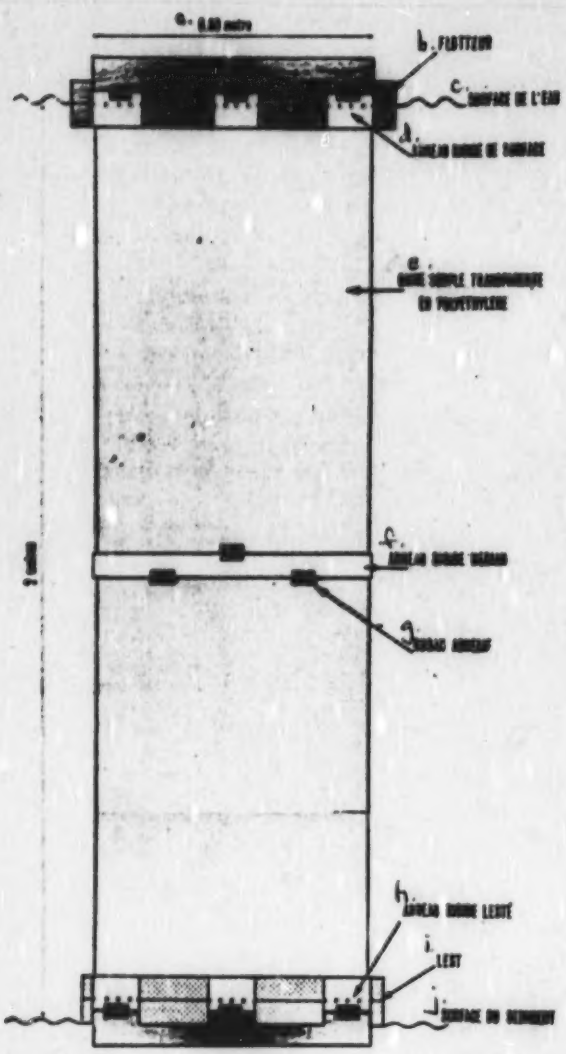

Fig. 1. Device used for in situ study of the effects of experimental pollution.

Key:
a. 0.60 m
b. Float
c. Surface of water
d. Rigid surface ring
e. Flexible transparent polyethylene sheath
f. Rigid median ring
g. Adhesive tape
h. Weighted rigid ring
i. Weight
j. Surface of sediments

1.596, is obtained by the action of phosphoric acid (46%) on sugar molasses (31%) in the presence of dicalcium phosphate (16%) and diammonium phosphate (7%). The nitrogen content of the Sefoil used in this study was 2.45%.

The Sefoil was used in a mixture of 100 parts hydrocarbon to 60 or 70 parts Sefoil, until an emulsion was obtained. The nutritive substances involved in its composition encourage the development of marine microorganisms and the rapid breakdown of emulsified hydrocarbons by these microorganisms.

Corexit 7664[1], with a density of 1.025, is composed of 27.23% oxyethylene sorbital oleate with 20 molecules ethylene oxide, 2.70% sorbital oleate, 5.92% isopropyl alcohol and 64.15% water. The amount of Corexit needed to treat and eliminate oil slicks varies with the nature of the hydrocarbons in the slick and the temperature of the water. It will be between 1 and 10% of the quantity of pollutants. This product has been very widely used for marine purposes over the last few years.

3) Measurements

The following list gives the factors studied, with the method used in parentheses:

-- salinity (Knudsen)

-- dissolved oxygen (Winkler)

-- chlorophyll a (Strickland and Parsons, 1965).

-- rate of photosynthesis (carbon 14 method: Steeman-Nielsen, 1952)

-- oxyethyelinated nonylphenol (Gref et al., 1965).

The water samples needed to measure the rate of photosynthesis, the chlorophyll a concentration, and the dissolved oxygen ratio, the pH and the

[1] Esso.

salinity were taken at 7:00 a.m. at a depth of 50 cm in the center of each device, with the use of a Wab handpump.

For the oxygen measurements, the water sample without contact with the air was immediately decanted into a 250-ml sample flask, into which the two reactants necessary for measurement were introduced. This initial phase of the measurements was performed on land. The second phase, which consisted in acidifying the environment and releasing potassium iodide iodine, was performed in the laboratory during the day.

The dry weights of the plankton from the water samples were obtained by filtering a set quantity of water through an HA 0.45 ± 0.02 µm millipore filter. The filter was subsequently dried at 110°. The samples were weighed before and after filtration.

For the carbon 14 method, the samples were incubated in the laboratory for 8 to 13 h in 125-ml ground flasks into which 3 µCi of $C^{14}O_3HNa$ were injected. We used a fluorescent tube battery yielding a luminous intensity of 6000 lux in the flasks. The samples were incubated at the temperature of the water measured prior to sampling.

After incubation, the water in each flask was filtered through a filtering membrane[1] 35 mm in diameter with 0.45 µm openings; the filter was then dried in a silica gel chamber. The count was made by means of a Geiger counter with thin terminal window. All the values obtained were deduced from the background and carbon was fixed in darkness for each test in "dark" flasks duplicating the "illuminated" flasks.

[1] L.M.R. No. 6, Labo Moderne, 37 rue Dombasle, Paris 15.

The photosynthetic activity of the water from the polluted ecosystems was expressed as a percentage of the photosynthetic activity of the water in the control ecosystem.

The method given by Greff et al. (1965) was used to measure surface-active oxyethylenes. This method is based on formation of a blue compound between the oxyethylenated chains of the alkylphenols and a reactant with ammonium cobaltothiocyanate which will precipitate out in a saturated NaCl solution and can be extracted by benzene; the optical density of this reactant was measured at 320 μm.

III. Results and Discussion

The rate of photosynthesis was measured 1, 3, 7, 10 and 15 days after pollution. The results, given in Fig. 2, permit a comparison of the effects of the three anti-petroleum products, Gamosol (surface-active part), Sefoil and Corexit.

1) Surface-Active Products in Gamosol

These products were found to be particularly harmful. We observed a total inhibition of photosynthesis over almost the entire experimental period (Fig. 2). The measurements of oxyethylenated surface-active compounds performed on water from the polluted ecosystem indicated a sharp degradation of these compounds, since their concentration was only 4 mg/ℓ at the end of the experiment (Table 1). Nevertheless, this concentration was still high enough to slow the photosynthesis of planktonic micro-algae considerably, as indicated by a second type of test performed at the same time. We felt it would be useful to determine the minimum concentration at which one of these surface-active agents in Gamosol -- nonylphenol with nine ethylene oxides -- would have an effect on photosynthesis under natural conditions.

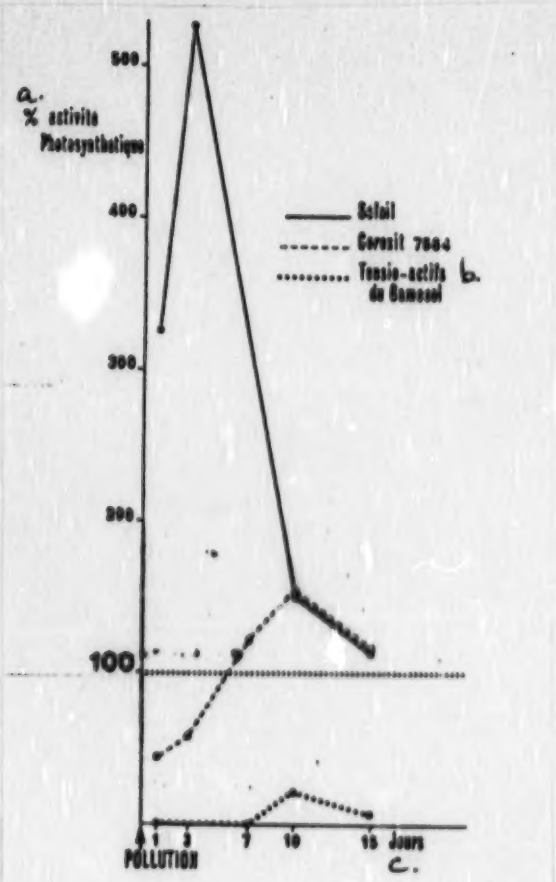

Fig. 2. Comparative effect of three anti-petroleum emulsive agents on the photosynthetic activity of ecosystems in situ. These results are expressed as a percentage of the photosynthetic activity of a control ecosystem.

Key: a. % photosynthetic activity
b. Gamosol: surface active part
c. Days

Four experimental devices of the type described above were given doses of 1, 5, 10, and 20 mg/ℓ of surface-active agent, respectively. Four days after pollution (Fig. 3), a sharp decrease in the rate of photosynthesis was observed for low surface-active product concentrations; thus a concentration

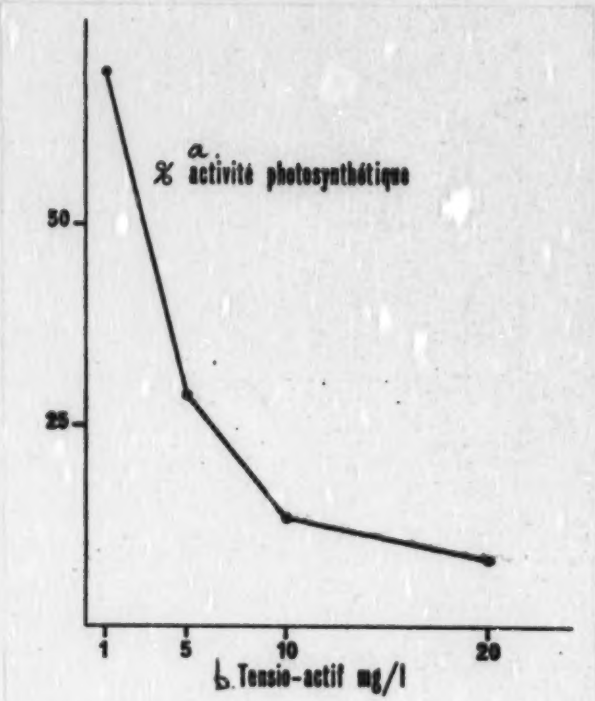

Fig. 3. Effect of Syntopon C (nonylphenol with nine ethylene oxides) on the photosynthetic activity of four ecosystems in situ. The results are expressed as a percentage of the photosynthetic activity of a control ecosystem.

Key: a. % photosynthetic activity
b. Surface-active part, mm/ℓ

of 1 mg/ℓ surface-active product decreased photosynthesis by 30%, while a concentration of 5 mg/ℓ produced a decrease of 75%. Beyond 10 mg/ℓ, photosynthetic activity was almost totally inhibited.

There are no significant differences in the pH and the dissolved oxygen concentration of the ecosystem polluted by the surface-active agents in Gamosol in comparison to the values present in the control ecosystem (Table 1). Diffusion to the surface of the enclosed water is obviously partially responsible for this. It may also be assumed that although the surface-active agents do decrease photosynthesis, as we have seen, they do

Table 1

Chlorophyll a (µg/ℓ) (1), dry weight (mg/ℓ) (2), pH (3), oxygen (mg/ℓ) (4), salinity (g °/oo) (5), radioactivity of filters (cpm) (6), oxyethylenated nonylphenols (mg/ℓ) (7)

	a. Jours écoulés après la pollution				
	1	3	7	10	15
b. Ecosystème témoin	2,57 (1) 19,86 (2) 8,05 (3) 7,82 (4) 22,43 (5) 6321 (6)	3,35 22,80 8,48 8,21 23,48 7501	2,14 21,10 8,25 7,79 26,09 3409	3,40 21,10 8,46 8,21 26,44 4160	5,95 17,70 8,87 8,83 26,61 8413
c. Ecosystèmes pollués : d.– tensio-actifs du Gamosol	1,91 (1) 23,00 (2) 8,24 (3) 7,02 (4) 39,90 (7)	2,14 – 8,31 8,19 –	0,00 18,70 8,20 7,78 28,80	0,00 21,00 8,47 8,01 –	1,87 21,30 8,85 8,39 4,00
– Sefoil	1,34 (1) 25,10 (2) 7,46 (3) 6,42 (4)	30,15 43,60 8,49 11,03	– 32,80 8,95 10,01	13,40 31,60 8,64 8,00	7,50 33,60 8,20 7,82
– Corexit	1,91 (1) – (2) 8,32 (3) 7,81 (4)	2,41 22,40 8,46 7,20	– 17,60 8,41 7,44	3,80 – 8,37 9,22	8,17 19,00 8,83 8,39

Key: a. Number of days after pollution
b. Control ecosystem
c. Polluted ecosystems
d. Surface-active products in Gamosol

[Note: Commas in numerals are equivalent to decimal points.]

not destroy very many organisms, or at least the biomass destroyed is small, and consequently the oxygen consumption is negligible. Thus one does not observe the low oxygen concentration generally resulting from the decomposition

of organisms killed by acute pollution, a phenomenon which generally virtually constitutes a secondary pollution (Bartch, 1967).

The in situ experiments described above were followed up by laboratory tests designed to determine the influence of several ecological factors. Water and sediments, with their related organisms, were removed from the same area where the in situ experiments were performed and were then taken to the laboratory. The sediments and the water, equally divided among large containers, thus made up experimental ecosystems which were subsequently placed under well-defined test conditions (Beyers, 1964). This type of ecological research has been termed the "microcosmic method" (Odum and Hoskin, 1957), due to the small size of the systems established (micro-ecosystems) in comparison to natural ecosystems and their isolation from the rest of the biosphere.

Several climatic chambers regulating illumination, photoperiod and temperature were used. It was thus possible to compare the influence of luminous intensity, photoperiod and temperature on the extent of the pollution and to evaluate the influence of these parameters on the degradation of nonylphenol with nine ethylene oxides (Syntopon C).

We were thus able to observe that the degradation of this product is obviously a function of temperature and the abundance of nutritive elements in the environment, and that it occurs just as slowly in illuminated ecosystems as in dark ecosystems: in light, the growth of microalgae slows the degradation of the pollutant, and there is probably competition between algal and bacterial microflora, resulting in less growth of bacteria which degrade petroleum.

The ecological effects of this observation should be noted: the extensive growth of algae resistant to the pollutant may limit the size of the bacteria populations responsible for its degradation. The factors which specifically check algae growth, such as turbidity, which disturbs normal light penetration, may also indirectly encourage the degradation of the pollutant.

Tests on the effects of oxyethylated nonylphenols have also been performed on planktonic algae cultures.

Thus Ukeles (1965) has tested the effects of oxyethylenated nonylphenols on 12 chlorophycean species of marine phytoplankton. There is a significant inhibition of culture growth beginning at 10 mg/ℓ. The products studied by Ukeles each had a predetermined number of ethylene oxides, ranging from nine to 30; a decrease in toxicity with an increase in the number of ethylene oxides was revealed.

We were able to make the same finding with the surface-active agents in Gamosol. The number of ethylene oxide molecules in the nonylphenols in Gamosol is between 4 and 9. The overall effect of these surface-active agents on cultures of the diatomate Phaeodactylum tricornutum is to produce a 50% decrease in growth for 8 mg/ℓ (Lacaze, 1969). We then performed the same experiment with nonylphenol with nine ethylene oxides alone, the compound in the Gamosol surfactant series with the most ethylene oxide groups attached: a 50% decrease in growth was obtained for 25 mg/ℓ.

It seemed of value to make a distinction between the lethal (algicidal) concentrations of nonylphenol with nine ethylene oxides and those which inhibit cell division even though living organisms are present (algistatic concentrations). Phaeodactylum was subjected to increasing pollutant

concentrations, up to 1 g. All the transplants were positive. Thus this product has an algistatic effect on this species of alga, even at extremely high concentrations, a fact which is worth noting if one wishes to take the mode of action of the surfactants into account in other cases.

Ukeles also revealed variations in toxicity depending on the group of chlorophyceans studied. He attributed these results to the fact that toxicity depends on the composition of the cell wall, which differs according to the taxinomic classification.

In our experiments, in two experimental laboratory ecosystems polluted with 40 mg/ℓ of this surfactant, we observed extensive growth of two Cyanophyceans on the walls: Phormidium tenue (Menegh.) Gomont and Anacystis marina Drouet and Daily.

Other studies were performed in the Plymouth laboratory (Smith, 1968) after the wreck of the Torrey Canyon. The surfactants tested were those contained in a highly emulsive agent, B.P. 1002, used at that time. Of the six species of phytoplankton considered, four species were destroyed with a concentration of 1.2 ppm. On the other hand, there was little effect on two species belonging to the genuses Dunaliella and Chlorella, both of them characteristic of brackish water plankton. Thus the ecological nature of the algae used for the test is extremely important. Due to their euryhalinity, these algae are probably better adapted to ion regulation; they are thus able to tolerate changes in cell wall permeability more easily than strictly marine species.

The effect of oxyethylenated nonylphenols was also studied in marine invertebrates. Research on the toxicity of a number of surfactants was recently begun with reference to three marine invertebrates: Scolelepis

fuliginosa, Capitella capitata, and Mytillus gallo-provincialis (Foret-Montardo, 1970). The test results in regard to nonylphenol with nine ethylene oxides (Syntopon C) are given in the table below (Foret-Montardo, personal communications). These results indicate that this product is toxic even at low concentrations. The average lethal dose (LD 50) is indicated here at 48 and 96 h. The concentrations are expressed in mg/ℓ (Table 2).

Table 2

DL 50 of Syntopon C (after Foret-Montardo)

	48 heures [a]	96 heures [a]
Scolelepis fuliginosa	1	0,1 – 0,5
Capitella capitata	1 – 2,5	0,1
Mytillus galloprovincialis [b] (eau polluée, mode calme)	5 – 10	0,5 – 1
Mytillus galloprovincialis [c] (eau pure, mode agité)	1 – 2,5	1 – 2,5

Key: a. Hours
b. Polluted water, calm state
c. Pure water, agitated state

The results obtained with Gamosol may be summarized briefly as follows.

The solvent fraction of Gamosol (86% of the product) is especially toxic, but its effects are of short duration due to the volatility of these solvents in dilute solution. The toxicity of the surfactant fraction (oxyethylenated nonylphenols; from 4 to 9 ethylene oxide groups) in regard to microalgae decreases as the number of ethylene oxide molecules increases. These products break down slowly in water (Chambon et al., 1967, 1968; Lacaze, in

preparation). Biodegradation is also a function of the number of ethylene oxide molecules: the higher this number, the slower the degradation. A number of ecological factors influence this biodegradation: temperature, illumination, photoperiod, abundance of nutritive elements in the water. Under natural conditions, the surfactants disturb the photosynthesis of phytoplankton, with sharp effects occurring with concentrations on the order of a milligram per liter. The effects of these products vary depending on the taxinomic groups of algae studied and their ecological nature; some algae are less sensitive to the pollutant due to their euryhalinity. These surfactants are also highly toxic to marine invertebrates.

2) Sefoil

In contrast to the Gamosol surfactants, Sefoil produces a strong and immediate increase in photosynthesis upon introduction into a marine environment. the rate becoming five times higher than that of the control ecosystem 3 days after the introduction of the product. Strong chlorophyll a concentrations rapidly appear in the ecosystem at the same time (Table 1). Nevertheless, the high rate of photosynthesis and the strong chlorophyll a concentration last for only a very short period of time. By observing the water in this ecosystem, we were able to note the presence of an abundant zooplankton consisting almost exclusively of copepods, which probably developed at the expense of a large fraction of the microalgae. The high dry weight of the plankton in this ecosystem at the end of the experiment thus probably corresponds to the biomass of the copepods. Here Sefoil acted as a fertilizer, producing a sharp increase in the biomass. The diammonium phosphate in Sefoil enters into solution and may explain the considerable growth of

microalgae. Additional tests were subsequently performed to compare the effects of the different components in Sefoil.

3) Corexit

The effects of Corexit were less marked than those of the other two anti-petroleum products described above. The water polluted with Corexit initially showed a decrease in photosynthesis of 50% in comparison to the control ecosystem over a period of a few days. Subsequently there was an increase in photosynthetic activity of 50% in comparison to the same control ecosystem.

IV. Conclusion

The results described above indicate that in situ experiments may provide a useful complement to laboratory tests and thus improve our knowledge of the actual effect of pollutants.

In order to develop a practical technique for in situ bioassays, it is necessary to conform to a few conditions which were met only partially in this preliminary work. Subsequent studies which we intend to perform will permit a gradual improvement in these techniques.

The makeup of an experimental device will obviously disturb natural conditions; these resultant changes are inevitable, but the device should be designed to reduce them to a minimum. In our experience, it is easy to detect the influence of isolating the water on the parameters analyzed. At the beginning and the end of the experiment, the control ecosystem will have higher chlorophyll a concentrations and, at the same time, will have assimilated a larger quantity of labeled carbon (Table 1). Dickman (1969) notes in this regard that the isolation of a quantity of water may result in a bloom.

To facilitate experimentation, it is necessary to construct a large number of identical ecosystems. By then introducing pollution in various concentrations, it is possible to determine the approximate threshold of this pollution.

Each device should also contain a large quantity of water so as to reduce the wall effect to a minimum (growth of undesirable organisms, primarily bacteria, on the walls). The value of using large volumes becomes obvious if one considers that the size of the population attached to the walls increases with the surface area/volume ratio (Strickland, 1966). In other words, the larger the container, the smaller the wall effect. For instance, the wall effect is generally high in the small flasks used for primary productivity measurements, with the result that the period of incubation must be reduced to a few hours.

Furthermore, with a large device it is possible to take frequent samples of significant size without changing the volume greatly. In our next experiments it would be desirable to take more samples -- every day, for example, and at different depths. An analysis of samples taken from the polyethylene wall and from the sediments might also furnish useful information. Thus it is virtually indispensable to increase the number of parameters analyzed, which almost inevitably will require the cooperation of specialists in various fields.

Other conditions must also be met in obtaining a practical method for in situ bioassays: despite their large size, the ecosystems must be simple, easy to handle, and low in cost, and it must be possible to build them rapidly.

The device described here is well adapted to a lagoon environment. Nevertheless, a number of other devices may be prepared as "models" of other types of ecosystems. As an example, large experimental basins have been constructed on shore (Edmonston, 1947, 1955; Odum et al., 1963; Pratt, 1950). In this case, the temperature and illumination conditions for the enclosed water must be the same as those of the natural environment. It is also possible to renew the water in these devices periodically and thus to reconstruct natural movements of the water (models of estuaries or intertidal zones, for example). It is also to advantage to excavate several similar basins (Abott, 1968; Whitworth and Lane, 1969).

We hope that the simple and inexpensive device which we have described here will aid experimental studies on pollution in aquatic ecosystems, since in situ research is indispensable in establishing a final estimate of the real toxicity of a pollutant.

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